Restored antioxidant circulating capacities in AIDS west african patients receiving an antioxidant nutraceutical *Cucumis melo* extract rich in superoxide dismutase activity (GliSODin®).

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Summary.

Patients with acquired immunodeficiency syndrome (AIDS) present a variety of pathologic alterations that are related to oxidative stress. Previous studies have reported a reduction in the circulating antioxidant status in these patients associated with a higher production of free radicals. In the present double blind clinical investigation, the effect of the oral supplementation by a plant superoxide dismutase extract embedded (GliSODin®) or not with gliadin was evaluated in 35 patients with AIDS (< 250/mm^3 CD4+ T lymphocytes) that did not received any anti-retroviral therapy. The control group consisted in 30 non HIV-1-infected patients. Compared to the control group, circulating erythrocytes Cu/Zn-superoxide dismutase (SOD1) activity and total antioxidant status were significantly ($p < 0.01$) decreased in AIDS patients and correlated with the increased plasmatic concentration of β_2-microglobulin that reflect the activation state of macrophages. Patients receiving the GliSODin® supplementation during 21 days normalized their circulating SOD1 activity and total antioxidant status and reduced their circulating levels in β_2-microglobulin indicating a correlation between the reduction of oxidative stress and the reduction of macrophage activation. Even whether this antioxidant restoration is not associated to an improvement of the immune status or a reduction of viral load this study demonstrate that the GliSODin® supplementation could regulate the activation state of macrophages and down-regulate the oxidative stress caused by the infectious process. In addition, by improving the antioxidant defenses in AIDS patients we prepare them to a better compliance and possibly to efficacy of the anti-retro viral therapy.

The acquired immunodeficiency syndrome (AIDS) is associated to an important of oxidative stress[1], that is, at least in part, responsible to the development of pro-inflammatory disorders and organ failures[2]. Even whether the development of the highly active antiretroviral therapy (HAART) reduced considerably the viral load the oxidative biological disorders are still present. In addition, the usual anti-retroviral therapies also induced important oxidative disorders in inducing mitochondrial swelling[3], thus contributing to the development of the oxidative disorders in HIV-1-infected patients. Indeed, the cumulative pro-oxidant effects of the infectious process itself and the potential toxicity of the HARRT could be at the origin of most of the inflammatory and toxicological disorders observed in these patients.

The development of new nutritional antioxidant formula[4] validated by human clinical trials open a new field of investigation in the prevention and treatment of oxidative disorders including these observed in AIDS patients. In the present randommized double blind clinical study we have evaluated the effect of oral supplementation with a nutritional *Cucumis melo* extract rich in superoxide dismutase activity (USA Patent # 6,045,809, and B. Dugas presentation at the SFRR congress, July 2002. Dugas, B. 2002. GliSODin®: a nutraceutical product that promote the oral delivery of superoxide dismutase. *Free. Radic. Biol. Med.(Abstract)* **33**: S64). This nutraceutical product, GliSODin®, is a 100 % vegetable product composed of: 1) a specific *Cucumis melo* extract callibrated for its SOD activity associated to 2) wheat gliadin biopolymers that only protect the SOD activity from the digestive process but also promote the translocation of the SOD activity through the intestinal barrier (I. Ezpeleta et al. Preparation of Ulex europaeus lectin-gliadin nanoparticle conjugates and their interaction with gastrointestinal mucus. *Int. J. Pharm.* 1999. **191**:25-32).

In the present double blind randommized clinical trial, 35 AIDS patients (men and women of a median of age of 35 years, ranging from 25 to 45 years), that did not received any antiretroviral therapy were divided in three groups receiving *per os* for 21 days:

I. the placebo (n=12),

II. the non-protected Cucumis melo extract rich in SOD activity (1000 IU of SOD per day)(n = 12) and,

III. and (n = 11) the GliSODin® product (1000 IU of SOD per day) (n = 11).

In the present study we firstly demonstrated that the circulating antioxidant status and the level erythrocyte Cu/Zn-SOD (SOD1) of the AIDS population of west African patients (n=35) were significantly decreased ($p < 0.01$ and $p < 0.001$ respectively according to the statistical Mann & Withney test) compared to non-infected healthy west African donors (Table 1). A limited but significant decrease in the glutathion-peroxidase (Gpx) activity ($p < 0.05$) was also

observed whereas the catalase activity was not affected. These data confirmed the existence of an important oxidative stress in AIDS patients not receiving any anti-retroviral therapy and suggested that the *per os* administration of nutritional antioxidant could be an important therapeutical supplement for these patients.

After 21 days of GliSODin® (1000 IU of SOD per day) *per os* supplementation, a significant restoration of the circulating antioxidant capacities was observed in AIDS patients ($n = 11$): $p < 0.01$ for the SOD1 activity, $p < 0.05$ for Gpx activity and $p < 0.001$ for the total antioxidant status. During the same period no significant effect were observed for the non protected *Cucumis melo* extract rich SOD activity and for the placebo (Table 1). These data demonstrated that only the GliSODin® preparation was able to efficiently support the antioxidant defenses in AIDS patients.

As already described this decrease in antioxidant defenses in AIDS patients was associated to an increased activation of macrophages as revealed by β_2-microglobulin measurements in the plasma (Figure 1A to 1C before treatments). In the present study we demonstrated that the GliSODin® *per os* supplementation (Figure 1C) significantly reduced the β_2-microglobulin plasmatic concentration ($p < 0.01$) after 21 days, whereas the placebo (1A) and the non-protected *Cucumis melo* SOD extract were not effective.

The correlation between this antioxidant restoration and the decreased β_2-microglobulin concentration in GliSODin®-supplemented AIDS patients suggested an effect of the GliSODin® product on the vital functions of macrophages (oxidative stress, regulation of inflammation, control of the immune functions....). However, this GliSODin® supplementation appeared not to affect neither the virus replication nor the immunological status (mainly CD4/CD8 expression) (Table 1). Based on these clinical data we confirmed the hypothesis that antioxidant supplementation in AIDS patients rather targeted the biological stresses induced by the infectious process rather than the virus replication itself or the immunological status of the patients. Indeed, increased circulating β_2-microglobulin concentration was described a strong predictor of macrophage activation in AIDS patients and was corrlated to the development pro-innflammatory disorders and especially those induced by TNF-α. So the reduction of the circulating SOD activity in AIDS west African patients strongly suggested that the consequent oxidative stress could be at the origin of most of the pro-inflammatory disorders observed in these patients. Indeed, several studies have demonstrated that the excess production of free radicals in HIV-1-infected patients not only played a role in the control of the viral replication but also was also an important biological factor in promoting not only lymphocyte apoptosis and immunosuppression but also pro-

inflammatory disorders and cancer[1,2]. Considering that the antioxidant impairement is an important side effect of the HIV-1-infectious process, many investigators have developed clinical trials using various antioxidant products (e.g. vitamin E, N-acetylcysteine etc.). The clinical data confirmed the potential interest of these antioxidant molecules but the clinical impact remained limited[5]. The development of new classes of functional nutritional products[4] (also called nutraceuticals) now allow a better functionalization of vegetal antioxidant. These nutraceutical products, which represent an important alternative of usual antioxidant drugs, could be used as therapeutical supplements in the HIV-1-infected population. The objective of these nutritional products is to normalize the production of free radicals and to protect cells and tissues against degenerescence and death. In conclusion to this clinical study we are able to demonstrate that a nutraceutical antioxidant product such as GliSODin® is able to restore a natural circulating antioxidant capacities and to reduce the activation state of macrophages in west African AIDS patients. This restoration of natural antioxidant defenses in AIDS west African patients suggest that these patients will be prepared to a better compliance and possibly of to a better efficacy of the anti-retro viral therapy[5].

References

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Legend to figure

Figure 1: Comparative β2-microglobulin concentrations in Ivory Coast AIDS patients treated with a Placebo (A), a free plant SOD extract (B) or with GliSODin® (C). The β2-microglobulin concentrations was measured in the plasma of AIDS individuals before and after the different kind of treatments (Placebo (A), the free SOD plant extract (B) and GliSODin®) using the nephelometric method on a Behring BN/100 (as described in P. Michel et al. J. Infec. Dis. 2000; 181:64-75)